

BIOCHEMISTRY, BIOPHYSICS,  
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## Comparison of the Functional Activities of Xanthine Oxidases Isolated from Microorganisms and from Cow's Milk

A. S. Gorbunova<sup>a</sup>, M. M. Borisova-Mubarakshina<sup>b</sup>, I. A. Naydov<sup>b</sup>,  
S. S. Osochuk<sup>c</sup>, and B. N. Ivanov<sup>b,\*</sup>

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**Abstract**—The characteristics of the formation of the superoxide radical anion ( $O_2^{\cdot-}$ ) and hydrogen peroxide by xanthine oxidases isolated from microorganisms and from cow's milk were investigated. The increase in pH led to an increase in the rate of xanthine oxidation with oxygen by both xanthine oxidases. The functioning of xanthine oxidase from milk along with the two-electron reduction of  $O_2$  to  $H_2O_2$  carries through the one-electron reduction of  $O_2$  to  $O_2^{\cdot-}$ , and the rate and the fraction of generation of  $O_2^{\cdot-}$  increased with increasing pH. Under operation of the microbial xanthine oxidase, the  $O_2^{\cdot-}$  radical was not detected in the medium. The results suggest a difference in the operation of active centers of enzyme from different sources.

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Xanthine oxidoreductase is a molybdenum-containing oxidoreductase widely spread in many animals, including humans [1, 2]. The main function of this enzyme is the oxidation of hypoxanthine to uric acid through the intermediate product, xanthine, which is an important component of purine catabolism. Xanthine oxidoreductase is present in cells in two forms—xanthine oxidase (XO) and xanthine dehydrogenase, with both forms being encoded by the same gene. Xanthine dehydrogenase uses  $NAD^+$  as an electron acceptor, whereas XO uses molecular oxygen. Oxygen reduction in the xanthine oxidation reaction in the presence of XO may lead to the formation of superoxide anion radical ( $O_2^{\cdot-}$ ) due to one-electron reduction of molecular oxygen ( $O_2$ ) and hydrogen peroxide ( $H_2O_2$ ) when two electrons are transferred to the

$O_2$  molecule [3]. The contribution of each process depends on the degree of reduction of the XO active center, which is composed of molybdenum, two Fe–S clusters, and FAD. The major product of  $O_2$  reduction is  $H_2O_2$  when these cofactors are completely reduced and  $O_2^{\cdot-}$  when they are oxidized [4]. These reactive oxygen species (ROS)— $O_2^{\cdot-}$  and  $H_2O_2$ —not only disturb enzyme functions and cause destructive modification of cell membranes but also perform protective and signaling functions in various diseases, including cancer [1, 5], and are involved in the defense against pathogenic microorganisms [6]. In medicine, the analysis of the activity and content of XO is used for diagnostic purposes as an index of pathological processes induced by ROS [13]. Xanthine/xanthine oxidase (X/XO) system is widely used as a source of ROS in the studies of the molecular mechanisms of ROS-induced processes. In addition, this system is standard for the evaluation of activity of superoxide dismutases (SODs)—enzymes catalyzing  $O_2^{\cdot-}$  dismutation [7, 8].

The main source of XO was animal milk (in particular, cow's milk) [9]. Starting from 1970s, microorganisms have begun to be used as sources of this enzyme [10]. According to published data, the opti-

<sup>a</sup> Kazan (Volga) Federal University, Kazan, 420008 Tatarstan, Russia

<sup>b</sup> Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow oblast, 142290 Russia

<sup>c</sup> Vitebsk State Medical University, Vitebsk, Republic of Belarus

\*e-mail: ivboni@rambler.ru; borpushchin@gmail.com